and RNA solely, in accordance with features of the present invention;

FIG. 1 C is a diagram of a column positioned inferior to a column-pressurizing device, in accordance with features of the present invention;

FIG. 2A depicts oxidative cleavage of DNA [is a reaction sequence of DNA labeling], in accordance with features of the present invention;

FIG. 2B depicts a DNA intermediate in a labeling sequence, in accordance with features of the present invention;

FIG. 2C depicts a DNA-based aldehyde in a labeling sequence, in accordance with features of the present invention;

FIG. 3A depicts an RNA-based lactone in a labeling sequence , in accordance with features of the present invention;

FIG. 3B depicts an RNA-based cross-linking substrate used to form labeled product, in accordance with features of the present invention;

FIG. 4 A-G is a depiction of the hybridizations resulting from operation of the invented column process ; and

FIG. 5 is a diagram of a gel matrix depicting the efficiency of bacterial labeling utilizing features of the present invention.

Page 16, line 27, change "Bs2" to - - BS2- -.

Page 17, line 22, change "second" to - -third- -.

IN THE DRAWING:

Please substitute the enclosed black and white formal drawing for the originally submitted color drawing for FIG 4. The new FIG. 4 is provided to be readily reproduced in black and white color. No new matter is contained in the drawing.

Please also substituted the enclosed FIG. 5 for the originally submitted FIG. 5. Originally-submitted FIG 5 lacks the crisp edges of the enclosed FIG. 5, primarily due to facsimile and photocopy artifacts. No new matter is contained in substitute FIG. 5.

Also provided is a two-buffer process for manipulating genetic material, the process comprising contacting cells containing the genetic material to a silica column; creating a first fraction of cell detritus and a second fraction containing the genetic material; confining the genetic material to the column; removing the cell detritus; subjecting the genetic material to radicals so as to produce reactive aldehyde groups on the genetic material; and attaching chromophore to the genetic material.

BRIEF DESCRIPTION OF THE DRAWING

The present invention together with the above and other objects and advantages may best be understood from the following detailed description of the embodiment of the invention illustrated in the drawing, wherein:

FIG. 1A is a schematic diagram of a column-based protocol for manipulating genetic material generally, in accordance with features of the present invention;

FIG. 1B is a schematic diagram of a column-based protocol for manipulating DNA and RNA solely, in accordance with features of the present invention;

FIG. 1 C is a diagram of a column positioned inferior to a column-pressurizing device, in accordance with features of the present invention;

FIG. 2A depicts oxidative cleavage of DNA, in accordance with features of the present invention;

FIG. 2B depicts a DNA intermediate in a labeling sequence, in accordance with features of the present invention;

FIG. 2C depicts a DNA-based aldehyde in a labeling sequence, in accordance with features of the present invention;

FIG. 3A depicts an RNA-based lactone in a labeling sequence , in accordance with features of the present invention;

FIG. 3B depicts an RNA-based cross-linking substrate used to form labeled product, in accordance with features of the present invention;

FIG. 4 A-G is a depiction of the hybridizations resulting from operation of the invented column process ; and

FIG. 5 is a diagram of a gel matrix depicting the efficiency of bacterial labeling utilizing features of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

A column-based protocol for manipulating genetic material is provided herein. The column can be used alone or in combination with any microarray system. A salient feature of such a system include the invented column employed for successive DNA/RNA isolation, fractionation, fragmentation, fluorescent labeling, and removal of excess free label and short oligonucleotides. To demonstrate the efficiency of the column protocol the inventors used microarrays of immobilized oligonucleotide probes whereby the microarrays are juxtaposed at a depending end of the column; and a portable battery-powered device for imaging the hybridization of fluorescently labeled RNA fragments with the arrays.

The inventors have utilized the invented column in the above-identified configuration for 16S ribosomal RNA identification.

The inventors have exploited a phenomenon that nucleic acids bind to silica in the presence of high concentration of salt. To eliminate all centrifugation steps, heretofore required in typical protocols, a syringe column configuration can be utilized. As a result of this syringe configuration (depicted in FIG. 1C), the invented column-based protocol requires only two buffers. The isolation of total nucleic acids or the fractionation of DNA/RNA is effected in 3 to 5 minutes, as opposed to the typical 60-120 minute procedures employing four or more buffers, as discussed, supra. The buffers utilized are those effecting lysis and binding of the target genetic material.

The entire mini-column procedure, from cell lysis to removal of excess fluorescent label, is executed within 20-30 minutes.

The mini-column combines a method of nucleic acid isolation utilizing guanidine thiocynanate, with a newly developed hydroxyl radical-based technique for DNA/RNA labeling and fragmentation. The chemistry of nucleic acid isolation and DNA/RNA fractionation is effected via the application of the two buffer system, outlined infra.